ACE2/ApoE double KO mice. These changes were associated with exacerbation of renal tubule ultrastructure injury and greater activation of Akt and ERK1/2 phosphorylated signaling. Conversely, treatment with hrACE2 significantly attenuated renal oxidative stress levels and ultrastructure injury, and prevented the expression of NOX4 and phosphorylated level of Akt and ERK1/2 in ApoEKO mouse kidneys. However, there were no changes in renal expression of NOX2 among groups.

CONCLUSIONS Deletion of ACE2 triggers greater increases in renal oxidative stress and tubular ultrastructure injury in the ACE2/ApoE double mutant mice with greater activation of Akt-ERK1/2 phosphorylated signaling. While ACE2 overexpression alleviates renal tubular injury in ApoE-mutant mice with suppression of superoxide generation and downregulation of the Akt-ERK phosphorylated signaling. Strategies aimed at enhancing ACE2 action may have important therapeutic potential for atherosclerosis and renal diseases.

GW26-e0204

Variants of Mitochondrial Genome in Patients with Subclinical Atherosclerosis

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OBJECTIVES Genetic predisposition plays an important role amidst the other risk factors in the development of atherosclerosis, a socially significant multifactorial disease. This study was aimed to identify the relationship between mitochondrial DNA (mtDNA) variants and the presence of subclinical atherosclerosis, which was defined ultrasonographically as the abnormal increase of intima-media thickness of common carotid arteries (cIMT).

METHODS For the accurate detection of mtDNA variants, high-throughput sequencing of the mitochondrial genome from blood leukocytes using the Roche 454 technology was carried out in 77 asymptomatic non-related subjects. To assess the state of the carotid artery wall, high-resolution B-mode ultrasonography was performed with ultrasound scanner SonoScape SSI-1000 (China) using a linear vascular 7.5 MHz probe. The borderline values for Russian population were used for detection of abnormal cIMT values.

RESULTS In patients with subclinical atherosclerosis, 70 mtDNA variants have been revealed that were characterized by the prevalence over 5% of the total sample or among patients or controls. Twenty-five of them occurred in coding region, and 5 were located in rRNA genes, 3 in tRNA genes, 5 were missense mutations. For 5 variants characterized by the increased frequency in healthy subjects there were significant differences from the patients with subclinical atherosclerosis. Three homoplasmic mtDNA variants were found only in patients with atherosclerosis. Six MtDNA variants were characterized by a more than 2-fold increased frequency in patients with subclinical atherosclerosis as compared to healthy subjects. As for 115 heteroplasmic mtDNA variants revealed, 15 were found both in healthy subjects and patients with subclinical carotid atherosclerosis. Three variants had higher frequency in healthy subjects, and two variants had higher frequency in patients with subclinical atherosclerosis.

CONCLUSIONS The data obtained in our study can be used to assess individual risk of atherosclerosis and for further studies on the role of mitochondrial genome mutations in the development of atherosclerosis and its clinical manifestations. The individual profile of certain mtDNA variants may partially explain atherosclerosis variability and genetic predisposition to atherosclerosis in population, which could be inherited by maternal line.

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GW26-e0218

Circulating lncRNA AC100865.1 from Monocytes as a Novel Biomarker for Coronary Artery Diseases

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OBJECTIVES Long non-coding RNAs (lncRNAs) have been found to be involved in coronary artery disease (CAD) development. Whether or not circulating lncRNAs work as a CAD biomarker, needs to be established.

METHODS Using microarray-based lncRNA expression profiling to explore the lncRNA expression in the circulating peripheral blood monocyte (PBMCs) and plasma from 15 CAD patients and 15 control subjects. After criteria (average normalized intensity is more than 7 with significance less than 0.005) based selection, and confirmed by quantitative RT-PCR. Using the analysis of the area under the curve (AUC) of the receiver operating characteristic (ROC) in large amount of population. Functional enrichment analysis was performed by GO and pathway analysis. Further validation using the specific siRNA in the THP-1 cell line, and the concentrations of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) in the culture media, were studied by ELISA.

RESULTS According to the array in the PBMCs and plasma, we found 86 lncRNAs that were differentially expressed in both PBMCs and plasma from 15 CAD patients and 15 control subjects. After criteria and confirmed by quantitative RT-PCR, only three lncRNAs (CoroMarker, BAT5 and IL21R-AS1) remained in the select candidate list. By ROC analysis, CoroMarker was found to be the best candidate biomarker for CAD with an AUC of 0.920, 95% CI 0.892-0.947. CoroMarker was independent from CAD risk factors and other cardiovascular diseases. In a prospective study, we found the sensitivity and specificity of CoroMarker were 76% and 92.5%, respectively. Functional enrichment analysis showed CoroMarker being clustered with small molecule metabolic process and the signal transduction signaling pathway. Further validation using the specific siRNA in the THP-1 cell line, the concentrations of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) in the culture media, were reduced significantly.

CONCLUSIONS The present study suggests that CoroMarker have an unanticipated role in inflammatory response to finally impact in the risk of CAD, and be a novel and specific functional biomarker for the diagnosis of CAD.

GW26-e1064

Captopril negatively regulated apamin sensitive SK channels in volume-overload rats

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OBJECTIVES Our previous study confirmed that in heart failure the expression and function of SK channels upregulated significantly and participated in electrical remodeling of cardiocytes, but the regulation of SK channels was poorly understood in this process. Studies indicated that the sensitivity of SK channels to $[Ca^{2+}]i$ is positively regulated by PP2A, which is activated by angiotensin II in many biological response paths. In the present study, we explored the effect of angiotensin converting enzyme inhibitor captopril on apamin sensitive SK channels in HF.

METHODS We used volume-overload induced heart failure rat model by aortocaval fistulas (HF group), and captopril was administrated by gavage (CAF group). Whole-cell patch clamp was performed to recording I_{KAS} (Apamin sensitive current), and I-V curve was applied to determine the the effect of captopril on I_{KAS}. We also explored the effect of captopril on the sensitivety of SK channels to $[Ca^{2+}]i$ by setting various $[Ca^{2+}]i$ (10, 100, 500, 900,10,000 nM) at single isolated cells, and the Hill equation ($y=1/[1+ (EC50/x)^n]$) was employed to fitted currents data(EC50 represents the $[Ca^{2+}]i$ concentration at halfmaximal activation of I_{KAS}, n represents the Hill coefficient). And immunofluorescent staining, real time PCR, western blot were also carried out to furtherly investigate the underlying molecular mechanism of the regulation.

RESULTS Captopril significantly decreased the mean I_{KAS} density at 0 mV (CAF 5.40 ± 1.11 pA/pF, n = 6 cells from 5 rats vs HF 8.90 ± 1.79 pA/pF, n = 5 cells from 6 rats $P<0.05$) when $[Ca^{2+}]i$ at 900 nM comparing with HF group, and obviously shifted down the I-V curve. Similarly, the I_{KAS} density was markedly downregulated by captopril when $[Ca^{2+}]i$ at 500, 1000, 10000nM (500 nM: CAF 4.21 ± 0.16 pA/pF, n = 6 cells from 4 rats vs HF 7.98 ± 0.43 pA/pF, n = 6 cells from 6 rats $P<0.05$; 1000nM: CAF 5.51 ± 0.71 pA/pF, n = 6 cells from 6 rats vs HF 8.96 ± 0.51 pA/pF, n = 6 cells from 5 rats $P<0.05$; 10000nM: CAF 5.80 ± 0.52 pA/pF, n = 5 cells from 3 rats vs HF 9.02 ± 0.57 pA/pF, n = 5 cells from 4 rats $P<0.05$), the data of the Hill fitting showed the significant difference in EC50 values and the Hill coefficients among the three group cells (EC50 CAF 313 ± 17 nM, HF 231 ± 11 nM, $P<0.05$;

Hill coefficients CAF 2.14 ± 0.16, HF 2.53 ± 0.14, P<0.05). Consistently, the results of real time PCR and western blot demonstrated that captopril significantly downregulated the expression of apamin sensitive SK channels (SK3 mRNA: CAF 2.10 ± 0.9, n = 6 vs HF 8.40 ± 2.10, n = 6; SK3 protein: CAF 0.40 ± 0.07, n = 6 vs HF 0.56 ± 0.09, n = 6).

CONCLUSIONS Captopril significantly downregulated the sensitivity of SK channels to $[Ca^{2+}]i$ and the SK3 channels expression in HF, and reversed the SK channels remodeling.

GW26-e1586

AMPK attenuates proliferation of cardiac fibroblast via regulating TGF-β1/Smad pathways

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OBJECTIVES AMP-activated protein kinase (AMPK) exerts inhibitory effects on cardiac hypertrophy. However, the mechanism remains unclear. The aim of the present study was to investigate the effects of AMPK on angiotensin II (AngII) -induced proliferation of cardiac fibroblast and the mechanisms involved.

METHODS Proliferation of cardiac fibroblast was induced by angiotensin II (AngII). Cardiac fibroblasts were treated with the specific AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR, 0.5 mmol/L) and the specific AMPK antagonist Compound C (1 umol/L), and then stimulated with AngII (1 umol/L). Cell proliferation and the DNA synthesis were measured by MTT assay and EdU incorporation assay. TGF-β1 and Smad2, 3, 4 mRNA and protein expression was detected using Real-Time PCR and western blot analysis.

RESULTS Activation of AMPK by AICAR could inhibit AngII-induced proliferation of cardiac fibroblasts, manifesting decreased DNA synthesis and collagen production ($P<0.05$). Moreover, AngII significantly increased the mRNA and protein expression of TGF-β1 and Smad2, 3, 4 ($P<0.05$). AMPK activation markedly reversed the elevated TGF-β1 and Smad2, 3, 4 mRNA and protein levels ($P<0.05$). Furthermore, Treatment of proliferated cardiac fibroblasts with Compound C blunted the effects of AMPK on proliferation of cardiac fibroblasts and changes to the TGF-β1/Smad pathway ($P<0.05$).

CONCLUSIONS AMPK activation could attenuate proliferation of cardiac fibroblast induced by AngII, which may be due to the inhibition of TGF-β1/Smad pathways.

GW26-e4768

Improved Recovery After Myocardial Ischemic Infarction by Copper Supplementation

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OBJECTIVES Depressed angiogenesis due to ischemic injury leads to myocardial infarction. Copper (Cu) is involved in angiogenesis and ischemia causes copper loss in the heart. The present study was undertaken to test the hypothesis that Cu supplementation improves myocardial angiogenesis, leading to regression of myocardial ischemic infarction in Rhesus monkey model.

METHODS Coronary artery ligation was used to produce myocardial ischemia and the monkeys developed myocardial infarction 4 weeks after ischemia. A newly developed ultrasound contrast microbubble composed of Cu-albumin coated structure was used to specifically deliver Cu into the infarct area. The treatment was performed twice a week for 4 weeks.

RESULTS This procedure effectively increased Cu concentrations in the infarct area and activated the angiogenesis factors including vascular endothelial growth factor (VEGF), VEGF receptor-1 (VEGFR-1), and other relevant factors. Along with these changes, myocardial infarct size was significantly decreased and the density of myocardial microvessels was significantly increased. In addition, cardiac function was significantly recovered, as evidenced by increased ejection fraction (EF) values and decreased end-systolic volume (ESV) measured by echocardiography.

CONCLUSIONS This study thus demonstrated that Cu supplementation improved cardiac structural and functional recovery after ischemic infarction.

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GW26-e5402

Discovery of a new conduction substrate associated with atrioventricular node-anterior extension pathway

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OBJECTIVES The atrioventricular node(AVN) plays a role in conducting action potentials at an appropriate conduction velocity from atria to ventricles. Its complex anatomical structures and functional longitudinal dissociations are considered important in delayed conduction and AVN reentrant tachycardia (AVNRT). Inferior nodal extensions(INE) are part of the AVN. It is thought that these extensions may be involved in slow-pathway conduction and are part of the underlying circuitry that causes AVNRT. Some other conduction tissue shared the same origin layers with AVN distribute around the two valve annulus. The retroaortic node defined as the enlargement of this node-like tissue is located on the right side of the atrium parallel with the aorta. The potential electrophysiological function of these node-like tissues is still unknown. Understanding their detail anatomical structure, histological features and electrophysiological behavior are significant to clear the complex conduction characteristics of the AV junction.

METHODS Adult rats(n=6), mice(n=5) and rabbit(n=5) were used. Serial sections from the entire AV junction were obtained. Masson's trichrome stain was performed on AVN regions to assess for fibrous tissue. Three connexins(Cx) proteins including Cx43, Cx40 and Cx45 which dominate the electronic conduction of the heart and three main ion channels($Na_v1.5$, $Ca_v3.1$ and HCN4) participating the depolarization of myocardial cell were immunohistochemically labeled. Serial sections were used to reconstruct a 3D computational model of the anatomy of the AV junction which display the different histological features at different levels.

RESULTS There appears to be an anterior extension of the AVN which connect retroaortic node and AVN. The anterior node extension (ANE), compact node and INE express the same connexin isoforms. $Na_v1.5$ labeling was abundant in the atrial and ventricular myocardium. $Na_v1.5$ labeling presents at a reduced level in the compact node, ANE and INE. $Ca_v3.1$ and HCN4 expression were mainly expressed in the $Na_v1.5$ reduced area. Further, connections between the atria and inferior extension occur indirectly via small branches. However, this was distinct from the connection pattern that we observed between the atria and ANE, which was direct.

CONCLUSIONS We conclude that the retroaortic node connect with ANE forming ANE which suggests there would be direct electric conduction between them. Characteristics of these structures are conserved among various species including rat, mouse and rabbit as we had proven. ANE, AVN and INE have nearly the same electronic level and action potential level structure basic that highlight they would have the same conduction properties, but there are different connection patterns in atrium between them that suggests there would be the subtle conduction velocity difference after accept the impulse, which provide a new location and substrate of conduction that may present new insights on mechanisms underlying normal AV conduction and AVNRT.

GW26-e2274

AVE 0991, Nonpeptide angiotensin-(1-7) analogue, modulates cardiac hypertrophy via reducing oxidative stress

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OBJECTIVES AVE 0991, the nonpeptide angiotensin-(1-7) (Ang-(1-7)) analog, is recognized as having beneficial cardiovascular effects. However, the mechanisms have not been fully elucidated. This study was designed to investigate the effects of AVE 0991 on cardiac hypertrophy and the mechanisms involved.

METHODS Mice were subjected to aortic banding (AB) to induce cardiac hypertrophy. *After treatment with* AVE 0991 (20 mg·kg·day (-1)) for four weeks, indices of cardiac hypertrophy and heart function were measured by echocardiography, histological analyses and quantitative